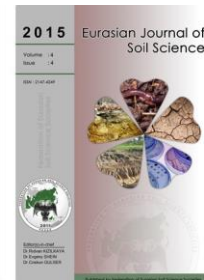




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Poultry feather wastes recycling possibility as soil nutrient

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Abstract

Poultry feathers are produced in large amounts as a waste in poultry slaughterhouses. Only 60-70% of the poultry slaughterhouse products are edible for human being. This means more million tons annually worldwide (Papadopoulos et al., 1986; Williams et al., 1991; Hegedűs et al., 1998). The keratin-content of feather can be difficultly digested, so physical, chemical and/or biological pre-treatment are needed in practice, which have to be set according to the utilization method. Feather was enzymatic degraded, and then fermented in separated bioreactors. The anaerobic bioreactor system (4 digesters with 6 litre volume) was controlled by ACE SCADA software running on Linux platforms. Pot scale seed germination tests were established to suggest the quantity of digested slurry to be utilized. The chosen test plants were lettuce (*Lactuca sativa*). In case of reproduction test Student's t-test was applied to examine significant differences between the root lengths of the control and the treated plant species. In case of pot seed germination variance analysis with Tukey B's and Duncan test was applied to examine significant differences between the root lengths of plants, grown on different treatments. The effect of treatments on germination ability of the plant species was expressed in the percentage of the controls. According to Student's t-test significant difference was found between root lengths of different treatments. Based on variance analysis with Tukey B's and Duncan tests could be detected a significant difference between the treatments. Utilization of the fermented material reduces the use of fertilizers and because of its large moisture content it reduces the watering costs. Recycle of the slaughterhouse feather and different agricultural wastes and by-products can solve three main problems: disposal of harmful materials, producing of renewable energy and soil nutrient, measuring reflectance at the certain spectral range, which can facilitate real time water status assessment of orchards.

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Introduction

The quantity of the agricultural products and food wastes was 1.188 thousand tonnes and from this was 14.2% energetically reutilized (KSH, 2008). The most commonly are utilized raw materials are manure, slurry, plant by-products, energy plants, but often is used food industrial by-products and wastes (Al Seadi et al., 2008). The most economical way of biogas production, when it is based on the secondary products and wastes, which are locally available (Tamás et al., 2006). Continuously and intensive growing of human population in the last 50 year in the world indicated a linear increasing of animal product demand. In 1967 was the ratio between the human populations and produced chicken 1:1. In 2012 this ratio changed to 1:3, so three times higher was the amount of chicken product than the number of human population in 2012 (Faostat, 2014). Parallel increased the amount of chicken slaughterhouse wastes, and feather wastes

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(Faostat, 2014). Feather is produced in large amounts and concentrate as waste in poultry slaughterhouses. Only 60-70% of the poultry slaughterhouse products is edible for human (Hegedűs et al., 1998). Dried feather contains 91% protein (Salminen and Rintala, 2002), the high protein content of poultry feather makes it an excellent raw material for biogas production. The keratin-content of feather difficult to degrade, therefore physical, chemical and/or biological pre-treatment are needed in practice, which have to be set according to the utilization method. Feather waste recycling with anaerobic digestion and utilization as soil nutrient provides an environmentally friendly way of utilization.

In case of soil contamination the natural physical, chemical and biological properties of the soil are changing enormously into a bad condition (Simon, 1998; Várallyay, 2007). The seed germination and root elongation technique was used because it is an easy and inexpensive screening test. Suggested plant species are cucumber, lettuce, radish, rice, sorghum, mustard, rape, turnip, cress, red clover and wheat varieties (Organization for the Economic Cooperation and Development (OECD), 1984); also oats, corn, cabbage, carrots, soybeans, tomatoes, ryegrass, onions and beans (United States Food and Drug Administration (USFDA), 1984; United States Environmental Protection Agency (USEPA), 1996).

Our main objectives were determine the methane potential of pre-treated feather waste and the most effective treatment ratios and examined the effect of solid digestate as soil nutrient. In case of reproduction test variance analysis with Tukey B's and Duncan test was applied to examine differences between the control and different treatments. The effect of treatments was expressed in the percentage of the controls. Utilization of the fermented material reduces the use of fertilizers and because of its large moisture content it reduces the watering costs.

Material and Methods

Batch digestion experiments

The anaerobic degradation was examined in the Biodegradation Laboratory of the Institute, where the fermentation areas were 4 stainless steel digester (the volume was 6 l per each) in Incubators. The batch experiments were carried out at mesophilic conditions (38°C) 30 day. Mesophilic liquid digestate (2.2 kg), corn silage (0.2 kg), cattle slurry (2.6 kg) and pre-treated feather (0, 5, 10, 20%) was added to the batch digesters (Figure 1). All experimental setups were performed in triplicates.



Figure 1. Batch anaerobic digestion with pre-treated chicken feather

The inoculum (Liquid digestate) was obtained from a large Hungarian Agricultural Biogas Plant that operating mesophilic and thermophilic condition. Slaughterhouse poultry feather wastes were collected from a local slaughterhouse industry. Before the microbial pre-treatment (*Bacillus licheniformis*) of feather were the samples sterilized at 100°C for 30 minute with Raypa type autoclave. Before the analysing the carbon and nitrogen-content of the feather with Elementar VARIO EL universal analyser, the sterilized samples were dried and ground into 2-5 mm particle size (Grinder: WARING®, Snijders). Dry and organic matter content was determined under standard method (Mézes et al., 2014). C- and N-content of biodegraded feather was calculated under keratin degradation ratio (%) (Mézes et al., 2014) and solubilisation degree (%) (Forgács et al., 2013).

Quantity parameters of raw materials were measured before setting up experiments, and after that was calculated a weighted average of DM% and oDM% by each the experiments (Table 1). The highest DM and oDM% was calculated by the control experiment without pre-hydrolysed feather.

Table 1. Average DM and oDM% by different experiments

Digester number	1	2	3	4
Pre-hydrolysed feather%	0	5	10	20
DM%	3.9±0.5	3.7±0.6	3.6±0.4	3.4±0.5
oDM% in DM%	3.1±0.4	2.8±0.3	2.5±0.2	2.3±0.2

Controlled thermometer probes (Pt100) and ventilators to ensure the optimal conditions in the incubator. The bioreactor system was controlled by ACE SCADA software running on Linux platforms which granted pre-programmed measurement and points of intervention for pH, temperature, CH₄, CO₂, O₂. The produced biogas flows through a safety gas-washer bottle and a cryogenic inventory. Following this the produced biogas was switched, so the producing line continues through a doubled valve-system or went directly to the output pipe. The gas-washer bottle was utilized to remove the organic acid while the cryogenic instalment to remove water (Tamás et al., 2012). The content of the gas mixture was monitored with custom created gas-analyser (CH₄, CO₂, O₂) and with MX42A gas-analyser (H₂S, NH₃) basis of absorbance measurement. The data transfer was achieved with serial RS232 port. During the anaerobic digestion process were analysed pH, pH_{KCl} (0.1 N), redox potential (ORP) (mV), conductivity (mS*cm⁻¹), total dissolved solid (g*l⁻¹), NaCl%-content of the liquid digestate samples with Hanna Instrument HI2550 multifunctional (pH/ORP/temperature/EC/TDS/NaCl) device. After the digestion process liquid and solid digestate have been separated.

Seed germination test, root elongation test and soil analysis

The chosen test plant for seed germination test and root elongation test was lettuce (*Lactuca sativa*) – Május királya (King of May), which has a seed germination time of 10 days in soils (Greene et al., 1989) with natural illumination. Well germinating lettuce seeds were placed into plastic pots, containing 1 dm³ soil and solid digestate mixtures. The rate of solid digestate were 0, 1, 1.5, 2, 5, 10, 20 V*V-1 %. Each treatment was conducted with three replicates. The control was sand soil and quartz sand (50:50 V*V-1 %). Irrigation was carried out daily, based on the quantity of evaporation. 20 seeds were germinated in one germination pot in a greenhouse at room temperature (20±2°C). Nutrients for plants were provided by the solid digestate. The lengths of the roots were measured on the 10th days. Within the assessment, the 3 lowest value out of 20 seedlings were not taken into account. The average length of the 17 germs was expressed in the percentage of the controls (Table 2).

Table 2. Toxicological qualification of the germs (Németh, 1998)

Average root length in the percentage of the control	Qualification
0 – 5%	extremely poisonous
6 – 50%	poisonous
51 – 90%	slightly poisonous
91 – 120%	non-poisonous
>120%	stimulating

The lettuce (*Lactuca sativa*) seeds were occurred on sterilized filter papers inside the Petri dishes at 20±2°C in incubator for 6 days. Twenty seeds were put into each dish. The rate of liquid digestate were 0, 5, 10, 25, 50, 100 V*V-1 %. Deionized water was applied by the control experiments. Each treatment was conducted with three replicates. The germination capacities in each treatment were expressed as percentage (%) of the control, root length was evaluated on the basis of Németh's qualification. Seed vigour index was calculated by multiplying germination (%) and seedling total length (mm) (Abdul-Baki and Anderson, 1973).

Statistical analysis

SPSS v14 statistical software (SPSS Inc., Chicago, IL, USA) were applied. In order to test the normal distribution of the data Kolmogorov-Smirnov test was used. Among root lengths results of each treatment Student's t-test (p<0.05) and variance analysis with Tukey B's and Duncan test were applied were applied to examine significant differences between the control and different treatments by 5% significant level, after proving the normal distribution of the root lengths belonging to one treatment, furthermore statistic indexes (mean, deviation, etc) were applied. The germination ability of the seeds was examined in the percentage of the control in each treatment on the 10th days.

Results and Discussion

Batch digestion experiments

The biogas raw materials were analysed before anaerobic degradation process (Table 3). Under these parameters were calculated the optimal DM, oDM content and C/N ratio of the digesters.

Table 3. Quality parameters of biogas raw materials

Raw materials	Corn silage	Cattle slurry	Liquid digestate	Pre-treated feather
DM%	26.0±2.73	3.6±0.52	2.8±0.91	19.94±1.36
oDM %	93.0±3.35	82.7±3.89	72.4±4.02	96.4±2.31
C:N ratio	27.6	13.0	18.0	1.47
C-content	45.8±0.87	40.4±2.40	47.3±2.07	15.09
N-content	1.7±0.29	3.1±1.21	2.6±0.25	10.26

The tables shown the pH values of the separated liquid and solid digestate after the anaerobic digestion process (Table 4, 5), which were used in germination tests. Between the pH values and ORP (mV) was not detectable a significant difference by the liquid and solid digestate after 30 day.

Table 4. Quality parameters of separated solid digestate

Solid digestate	pH	pH _{KCl}	ORP (mV)	Conductivity (mS*cm ⁻¹)	NaCl%	TDS (g*l ⁻¹)
1. Digester (0%)	8.8	8.6	-80.5	5.32	9.5	2.21
2. Digester (5%)	8.71	8.46	-70.2	5.82	10	2.57
3. Digester (10%)	8.82	8.34	-64.5	6.78	13.2	3.32
4. Digester (20%)	8.67	8.1	-58.4	5.47	10.4	2.72

Table 5. Quality parameters of separated liquid digestate

Liquid digestate	pH	ORP (mV)	Conductivity (mS*cm ⁻¹)	NaCl%	TDS (g*l ⁻¹)
1. Digester (0%)	9.85	-135	13.51	26.5	6.78
2. Digester (5%)	8.15	-56	27.88	54.4	13.99
3. Digester (10%)	7.79	-39.6	23.64	46.1	11.84
4. Digester (20%)	8.55	-75.2	15.55	30.4	7.76

After the anaerobic digestion process could be detected three times higher conductivity values (mS*cm⁻¹) and NaCl-content (%) in liquid digestate than solid digestate. In case of the liquid digestate the total dissolves solid (g*l⁻¹) values were also three times or four times higher than in case of solid digestate. No significant differences were detectable between the different treatments.

Biogas production of biodegraded chicken feather

Upon the results of the experiments it can be stated the mixture rate of the raw material that contains both cattle slurry and poultry feather determined the biogas production significantly (Figure 2).

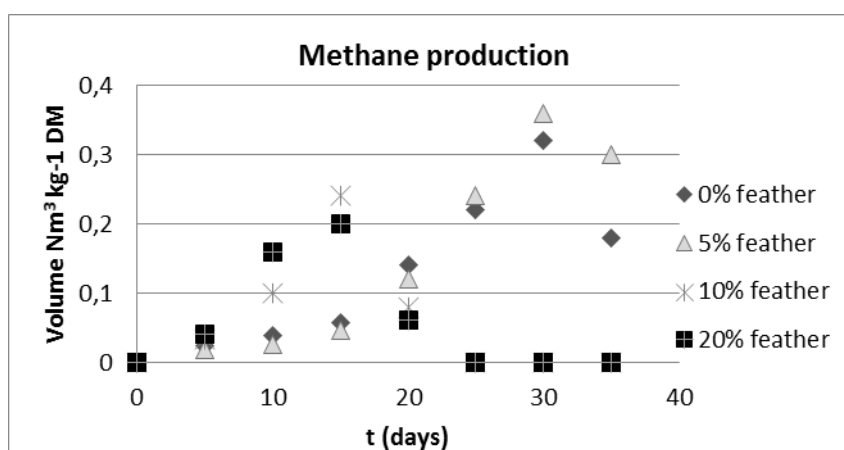


Figure 2. Methane production during co-digestion of different pre-treated feather ratio

Under mesophilic conditions the mixture rates of 5% result in a favourable production, the amount of the produced biogas (Nm³ day⁻¹) exceeded the values of the production at mixture rates of 10 and 20% by far (50%). The 5% mixture result the highest methane yield, the maximal value was 0.36±0.13 Nm³ kg⁻¹ DM. second was the control experiment (0.32±0.13 Nm³ kg⁻¹ DM) after 30 days. No significant differences could be detected between control and 5% experiments. [Forgács et al. \(2011\)](#) reported 0.35 Nm³ kg⁻¹ VS methane

production of feather after biological pre-treatment with *B. licheniformis* ATCC 53757 strain. Anaerobic digestion process of 10 and 20% mixture was stopped after 20 days despite the initial high biogas yields. Under methane yields two groups could be selected. Control, 5% experiments and 10. 20% experiments showed significant differences.

Biogas quality analysis

The biogas quality in case of the poultry feather mixture rate of 5 showed better results and differed significantly from the rates of 10 and 20%. In case of treatments with a feather mixture rate of 5 methane concentrations around 60% stayed stable (Figure 3).

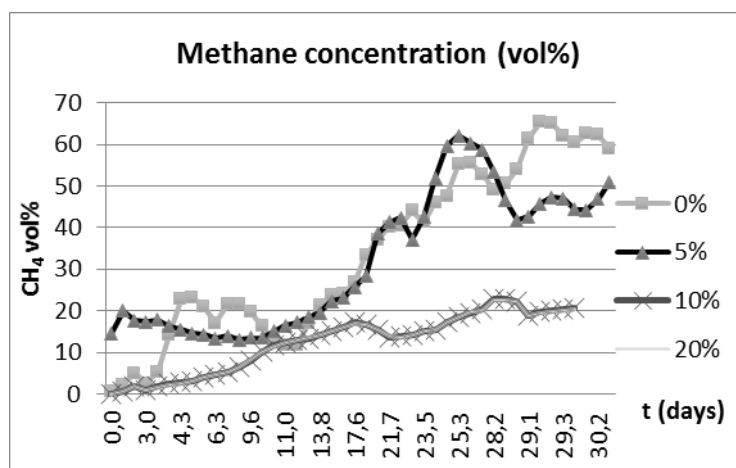


Figure 3. Methane concentration (Vol%) production during co-digestion of different pre-treated feather ratio

The amount of H_2S – that has a corrosive effect and causes bad smell – was significantly increased in case of a feather mixture rate of more than 10% (10 and 20%) at the beginning of the fermentation and it affected the methane production negatively. In case of the mixture rates of 5 the hydrogen sulphide concentrations of the produced biogas – in contrast to the higher mixture rates – were more favourable and showed a significant difference in the first phase of the production. The production of the hydrogen sulphide reached its maximal value already on the 9th day in case of the 10% treatment.

Regarding the ammonia content of the biogas it can be stated that the produced amount was significantly high in the first stage, because the most of the easily degradable nitrogen. After that this value decreased as the not so easily degradable forms were degraded. This process was more balanced. In the first stage of the ammonia production a significant difference could be revealed between the following groups: 0, 5% and 10, 20% treatments. In the much more balanced ammonia-producing final stage three groups could be differed: 0% treatment build the first, and 5% was the second, while the 10 and 20% were the third group. These treatments showed a significant difference.

Seed germination test, root elongation test and soil analysis

The data (N=295) shown normal distribution, the descriptive statistic shown 1.84 mean and 1.82 standard deviation in case of germinating test in plastic pots. Descriptive statistics of lettuce root lengths (mm) and Variance analysis with Tukey B's and Duncan test shown the Table 6, with Tukey B's test 10 different groups, with Duncan test 13 groups could be selected. More groups could be selected with Duncan test, therefore this test was applied to determine the significant differences between the treatments.

All the treatments possess normal distribution based on Kolmogorov-Smirnov test. The effect of different separated solid digestate could be detected using Variance analysis. Significant differences could be determined between the control test and other treatments. 13 different group was separated with the intermediate categories. Two mean groups can be distinguished addition the control treatments: less than 5% and more than 5% solid digestate applying (except the D4 1.5% treatment), but separation within the two mean group are not clear, therefore, further studies are needed.

During the evaluation of the results, the toxicological qualification of the germs was performed for each treatment on the basis of Németh's method (on the basis of the average root length in the percentage of the control) where 0-5% means extremely poisonous, 6-50% poisonous, 51-90% slightly poisonous, 91-120% non-poisonous, over 120% stimulating medium (Németh, 1998) (Table 7).

Table 6. Descriptive statistics and variance analysis of lettuce root lengths (mm)

Treatments	Mean	Std. Deviation	Minimum	Maximum	Groups (Duncan)	Legends
1	6.288	1.5708	3.6	10.3	h	Control
2	1.253	0.6345	0.3	2.5	cde	D2*1%**
3	1.747	0.6644	0.3	2.5	e	D3 1%
4	1.367	0.5367	0.1	2.0	de	D4 1%
5	1.638	0.7247	0.5	2.6	e	D2 1.5%
6	1.388	0.5894	0.5	2.6	de	D3 1.5%
7	1.082	0.6821	0.1	2.2	abcde	D4 1.5%
8	3.316	0.6295	2.1	4.5	fg	D1 2.5%
9	2.592	0.5838	1.9	3.5	f	D2 2.5%
10	2.625	1.5028	0.1	4.2	f	D3 2.5%
11	3.563	0.8269	2.0	4.8	g	D4 2.5%
12	0.400	0.4528	0.1	1.4	abc	D1 5%
13	0.282	0.2840	0.1	0.9	ab	D2 5%
14	0.682	0.5844	0.1	1.9	abcd	D3 5%
15	1.141	0.6305	0.3	2.2	bcde	D4 5%
16	0.525	0.4554	0.1	1.3	abcd	D1 10%
17	0.198	0.2458	0.1	0.7	a	D2 10%
18	1.166	0.8856	0.1	2.5	bcde	D3 10%
19	0.750	0.8983	0.1	1.9	abcd	D4 10%
20	0.325	0.1500	0.1	0.4	ab	D1 20%
21	0.450	0.0707	0.4	0.5	abc	D2 20%
22	0.458	0.4247	0.1	1.3	abc	D3 20%
23	0.406	0.4110	0.1	1.3	abc	D4 20%
F value***	55.426					

*D2= Number of Digesters **1%= Ratio of applied liquid digestate *** P < 0.001

Table 7. Toxicological qualifications based on the average root lengths in the percentage of the control

Treatments	Germination (%)	Average root lengths (mm)	Qualification (Németh, 1998)	Legends
1	85	6.52	non-poisonous	Control
2	85	1.25	poisonous	D2*1%**
3	85	1.75	poisonous	D3 1%
4	75	1.37	poisonous	D4 1%
5	80	1.64	poisonous	D2 1.5%
6	90	1.39	poisonous	D3 1.5%
7	85	1.08	poisonous	D4 1.5%
8	65	3.32	slightly poisonous	D1 2.5%
9	60	2.59	poisonous	D2 2.5%
10	60	2.63	poisonous	D3 2.5%
11	80	3.56	slightly poisonous	D4 2.5%
12	40	0.40	poisonous	D1 5%
13	55	0.28	poisonous	D2 5%
14	85	0.68	poisonous	D3 5%
15	100	1.14	poisonous	D4 5%
16	50	0.53	poisonous	D1 10%
17	30	0.20	poisonous	D2 10%
18	80	1.17	poisonous	D3 10%
19	30	0.75	poisonous	D4 10%
20	20	0.33	poisonous	D1 20%
21	10	0.45	poisonous	D2 20%
22	60	0.46	poisonous	D3 20%
23	40	0.41	poisonous	D4 20%

*D2= Number of Digesters **1%= Ratio of applied liquid digestate

On the basis of Németh's qualification poisonous, toxic effect have been determined by every treatments except of the control treatment, therefore in the future further experiments are needed.

The data (N=157) shown normal distribution in case of root length in case of Petri dishes using liquid digestate, the descriptive statistic shown 0.666 means and 0.4 standard deviations. Descriptive statistics of lettuce root lengths (mm) and variance analysis with Tukey B's and Duncan test shown the Table 8, with Tukey B test and with Duncan test also 4 different groups have been selected.

Table 8. Descriptive statistics and variance analysis of lettuce root lengths (mm) in Petri dishes

Treatment s	Mean	Std. Deviation	Minimum	Maximum	Groups (Tukey B)	Legends
1	1.056	0.3578	0.5	1.8	c	Control
2	0.860	0.3470	0.2	1.5	bc	D1*5%**
3	0.200	0.1414	0.1	0.3	a	D3 5%
4	0.422	0.2194	0.1	1.2	a	D4 5%
5	0.497	0.2845	0.1	1.5	ab	D1 10%
6	0.245	0.1036	0.1	0.4	a	D4 10%
7	0.567	0.3116	0.1	1.0	ab	D1 25%
F value***	20.45					

*D1= Number of Digesters

**5% = Ratio of applied liquid digestate

Control belongs in the first group based on Variance analysis with Tukey B's test. 5% liquid digestate ratio without pre-treated feather were selected as second group. Those treatments, for which pre-treated was used as raw material, were classified as third group and treatments without pre-treated feather used as raw material was classified as another group.

Germination capacities (%), root length (Németh's qualification) and seed vigour index shown the Table 9. in case of Petri dishes tests using separated liquid digestate as nutrient.

Table 9. Germination (%) and vigour index (VI)

Petri dishes test	Control	D1* 5%**	D3 5%	D4 5%	D1 10%	D4 10%	D1 25%
Germination (%)	100±0	88.24±16.6	11.76	67.65±12.48	97.06±4.16	32.35±1.48	70.59±8.32
Total seedling length	2.37±0.72	4.04±0.48	1.20	2.15±0.05	3.46±0.09	1.7±0.14	4.08±0.80
Vigour index	237.06±72.37	352.65±24.5	14.12	145.59±30.36	336.18±23.7	55.88±25.79	284.41±22.88
Qualification (Németh, 1998)	non-poisonous	slightly poisonous	poisonous	poisonous	poisonous	poisonous	slightly poisonous

*D1= Number of Digesters

**5% = Ratio of applied liquid digestate

The highest germination was observed in the control experiments followed by 10% solution without pre-treated feather. No germination was detectable, when the liquid digestate ratio was higher than 50% and the pre-treated feather ratio was higher than 10%. Highest shoot and root growth were observed in case of 25% liquid digestate, followed by 5% and 10% liquid digestate without pre-treated feather. Vigour index was highest in case of 5% liquid digestate without pre-treated feather added, followed by 10% liquid digestate without pre-treated feather added and was significantly different from control (Table 9). Poisonous effect could be detected by 5% liquid digestate ratio applying pre-treated feather based on Németh's qualification. Slightly poisonous could be determined by using 5% liquid digestate ratio without pre-treated feather.

Conclusion

In our case 5% pre-treated feather ratio result in a favourable biogas production, the amount of the produced biogas (Nm³ day⁻¹) exceeded the values of the production at mixture rates of 10 and 20% by far (50%). The 5% mixture result the highest methane yield, the maximal value was 0.36±0.13 Nm³ kg⁻¹ DM. second was the control experiment (0.32±0.13 Nm³ kg⁻¹ DM) after 30 days. Anaerobic digestion process of 10 and 20% mixture was stopped after 20 days despite the initial high biogas yields. Control, 5% experiments and 10, 20% experiments showed significant differences. Due to the amount of produced

hydrogen sulphide (ppm) the critical mixing ratio of feather proved to be 10% in laboratory environment. The production of the hydrogen sulphide reached its maximal value (200 ppm) already on the 9th day in case of the 10% treatment. Three treatments group could be selected in case of the highest ammonia concentrations (control and 5% and 10. 20%), which were detected after 20 days.

Germination tests in culture dishes containing standard quartz sand and sand soil (50:50 V*V-1 %) were carried out with separated solid digestate at room temperature. Germination test with liquid digestate was carried out in Petri dishes. The germs' root lengths (mm) were measured at the end of the experiments, and the Tukey's and Duncan test was used to specify the significant differences between the treatments. All the treatments possess normal distribution based on Kolmogorov-Smirnov test. In case of germinating test in plastic pots 13 groups could be selected with Duncan test. Significant differences could be determined between the control test and other treatments. 13 different group was separated with the intermediate categories. Two mean groups can be distinguished addition the control treatments: less than 5% and more than 5% solid digestate applying (except the D4 1.5% treatment), but separation within the two mean group are not clear, therefore, further studies are needed.

On the basis of Németh's qualification poisonous, toxic effect have been determined by every treatments except of the control treatment, therefore in the future further experiments are needed.

The highest germination was observed by Petri dishes tests in the control experiments followed by 10% solution without pre-treated feather. No germination was detectable, when the liquid digestate ratio was higher than 50% and the pre-treated feather ratio was higher than 10%, therefore lower rate than 10% of pre-feather should be applied as biogas raw material based on the germination tests. Due the pre-treated feather applying increased the shoot length significantly, which could be due the high nitrogen content of liquid digestate. Poisonous effect could be detected by 5% liquid digestate ratio applying pre-treated feather based on Németh's qualification. Slightly poisonous could be determined by using 5% liquid digestate ratio without pre-treated feather.

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